Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v16i2.3409
Article

Biological effectiveness of imidacloprid on the Asian citrus psyllid

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Abstract

Diaphorina citri Kuwayama, better known as the Asian citrus psyllid, is the main pest of the Persian lime due to the direct and indirect damage it causes and, currently, there are not enough chemical insecticides for its control in the field. Therefore, this work aimed to determine the biological effectiveness of 12 successive applications of imidacloprid at 25-day intervals on immature Asian citrus psyllid individuals in a Persian lime grove in order to define whether the effectiveness of the pesticide decreases as a function of the number of sprays. Before applying the insecticide, 18 tender shoots were sampled and 72 h after application, the number of live immature *D. citri* individuals was counted. A completely randomized design was used and mortality data were subjected to an analysis of variance. The biological effectiveness of imidacloprid on *D. citri* was high (up to 90%) during the first successive applications, then mortality on eggs declined by 10% in the sixth application, whereas in the tenth, it decreased significantly by more than 30%. In small nymphs, the reduction in mortality was 10% from the seventh application and in the tenth, it was significantly lower (17%). In large nymphs, the reduction in mortality was 10% in the seventh application while in the eleventh, it significantly declined (18%). In conclusion, the biological effectiveness of imidacloprid decreased after several successive applications on immature *D. citri* individuals.

Keywords:

chemical control, insecticide, mortality.

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Introduction

Citrus are grown in approximately 50 countries, which are mainly located in tropical and subtropical regions (Ladaniya *et al*., 2019). Global citrus farming has great economic, food, and industrial importance and because of this, it is necessary to consider various strategies that maintain or increase this productivity (Mattos, 2018).

Currently, Mexico is considered one of the largest citrus producing countries in the world, 8.6 million tonnes are produced annually. Within the citrus fruits, the Persian lime *Citrus latifolia* Tanaka (Rutaceae) stands out because it is the only one that is exported fresh (18 000 t); it is extremely important as it is used for fresh consumption and industrial use (export as concentrated juice and essential oil) (Arias and Suárez, 2016; Hernández and Botello, 2017; Borja *et al*., 2021; San Martin *et al*., 2021; SIAP-SAGARPA, 2022).

Of the main biotic factors that cause direct and indirect damage to Persian lime crops, more than 40 pathogens and pest insects have been reported in Mexico alone, causing a reduction in production, fruit quality, and plant death (SADER, 2021).

The main pathogens are *Rhizoctonia solani* (Basidiomycota), *Pythium* sp. (Oomycota), *Phytophthora* spp. (Oomycota), *Colletotrichum gloeosporioides* (Ascomycota), *Capnodium citri* (Ascomycota), *Candidatus* Liberibacter asiaticus (Proteobacteria), citrus tristeza virus (Closteroviridae), citrus leprosis virus (Rhabdoviridae), and wood pocket (physiopathy); on the other hand, the main arthropod pests are citrus red mite *Panonychus citri* McGregor (Acari: Tetranychidae), citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), various aphids, such as the green citrus aphid *Aphis spiraecola* Patch, cotton aphid *Aphis gossypii* Glover and the black citrus aphid Fonscolombe Boyer (Hemiptera: Aphididae), citrus blackfly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae), citrus flat mite *Brevipalpus* spp. (Acari: Tenuipalpidae) and *Diaphorina citri* Kuwayama (Hemiptera: Liviidae).

The latter is the most economically important in the region due to the direct and indirect damage it causes (vector of the HLB disease, huanglongbing) (Rodríguez, 2002; Flores *et al*., 2015; García and Cortez, 2020; Cortez *et al*., 2021; SADER, 2021). The life cycle of *D. citri* lasts approximately 16 days under optimal development conditions (25 °C, 70% relative humidity, and 12 h photoperiod). The eggs are deposited in the tender shoots, are ovoid in shape and have an elongated prolongation at the top, are yellow and turn orange as they develop, and are approximately 0.3 mm long and 0.12 mm wide.

They go through five nymphal stages: nymph 1 (N1), they measure between 0.24 - 0.32 mm in length and 0.1 - 0.18 mm in width, are yellow-orange in color and the compound eyes are red, they have small antennae; nymph 2 (N2), they are recognized because they have developing wing buds, the antennae are white and have a black spot on the apical part, the compound eyes are red and the nymphs are the same color as N1, their size ranges from 0.47 - 0.5 mm in length and 0.27 - 0.3 mm in width; nymph 3 (N3), they are yellow-orange, the eyes are red, the wings are more developed (although not completely), the antennae are black, and their size ranges from 0.95 - 1 mm in length and 0.71 - 0.75 mm in width; nymph 4 (N4), they have almost fully developed wings, the eyes are also red, like the previous nymphal stages, and the body is yellow, they are quite mobile, and their size is about 1.4 - 1.52 mm in length and 1.09 - 1.13 mm in width; nymph 5 (N5), they measure between 1.61 - 1.66 mm in length and 1.1 - 1.12 mm in width, the wing buds are very developed, they are very mobile, the antennae are black, their body is yellow, and the eyes are red.

Adults are recognized by the resting position, they sit at an approximate angle of 45 degrees, have wings with brown spots, the eyes are red and the antennae are black, the head and thorax are brown while the abdomen is bluish-green, they are between 2.24 - 2.3 mm long and 0.61 - 0.65 mm wide and adult flight is short (less than one meter away) (Alves *et al*., 2014; García *et al*., 2016).

Currently, Mexico is implementing a strategy that is unique among the other citrus cultures of greater importance in the rest of the world because the management of *D. citri* is carried out through periodic regional spraying of chemical and biorational insecticides in strategic management units commonly known as regional control areas (ARCOs, for its acronym in Spanish) (Mora et al., 2013; Robles et al., 2016; SENASICA, 2017; Cortez et al., 2021).

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Several authors have described the resistance of *D. citri* to chemical insecticides, such as García *et al*. (2019), who monitored the resistance of this insect in four commercial formulations (cypermethrin, chlorpyrifos, dimethoate, and imidacloprid) in five ARCOs in Mexico and obtained resistance proportion percentages of 11.5, 83.25, and 79.72, respectively (imidacloprid did not generate resistance). Another study by Sánchez-Ramírez *et al*. (2023) showed levels of resistance in adults of *D. citri* (12%) to imidacloprid.

By contrast, Naeem *et al*. (2023) found that imidacloprid showed a very high level of resistance in twelve *D. citri* populations collected in the field. Likewise, research from various parts of the world has documented the development of resistance to imidacloprid in different insects (Kaufman *et al*., 2010; Memmi, 2010). Therefore, it can be concluded that imidacloprid has shown cases of resistance on some populations of *D. citri*.

The effect of imidacloprid has also been evaluated on non-target organisms, as in the case reported by Luna-Cruz *et al*. (2011), who mention that this insecticide was the least aggressive on adults of the parasitoid *Tamarixia triozae* (Hymenoptera: Eulophidae) compared to the other insecticides evaluated (azadirachtin, spinosad, and abamectin); Fernández and Giménez *et al*. (2005) found that the biological activity of soil decomposers is not affected by the application of imidacloprid in foliar and soil applications.

A review by Gibbons *et al*. (2015) mentions that the use of imidacloprid as a seed treatment in some crops poses risks to small birds, and the ingestion of even a few treated seeds could cause mortality or reproductive deterioration in sensitive bird species. Although ambient concentrations of imidacloprid are at levels below those that would cause mortality in freshwater vertebrates, they can produce sublethal effects.

Neonicotinoids are capable of exerting direct and indirect effects on terrestrial and aquatic vertebrate wildlife, warranting further review of their environmental safety. On the other hand, according to Cortez *et al*. (2013, 2021), the number of chemical insecticides authorized (abamectin, mineral oil, acetamiprid, chlorantraniliprole, cyantraniliprole, fenpropathrin, fenpyroximate, flonicamid, flupyradifurone, diflubenzuron, spirotetramat, spinetoram, and thiamethoxam) for the control of ACP is limited.

Among them, imidacloprid is one of the most widely used due to its biological effectiveness and residuality, especially when applied through the drip irrigation system (Ahmed *et al*., 2004); it is a neonicotinoid in subgroup 4A, systemic, translaminar with stomach and contact action, but, according to the preventive management of resistance, as with other insecticides, it is necessary to use it rationally in rotations with other insecticides of different toxicological groups, depending on the mode of action and, if possible, using others with a different site of action (Cortez *et al*., 2013; IRAC, 2022).

In some countries, such as China, Pakistan, the United States of America, and Mexico, at least five cases of resistance of *D. citri* to the chemical insecticide imidacloprid have been detected (Vázquez *et al*., 2013; Naeem *et al*., 2016; Fajun *et al*., 2018; García *et al*., 2019), due to this situation, it is necessary for their use to be reduced in some regions of Mexico. In accordance with the above, this research aimed to measure the biological effectiveness of 12 successive monthly applications of imidacloprid on immature individuals of the Asian citrus psyllid *D. citri* in the field in order to define whether the effectiveness of the insecticide decreases as a function of the number of sprays applied.

Materials and methods

This work was carried out in a commercial Persian lime grove that was in a state of production, approximately three years old, located in the area near the locality of Higuera de Zaragoza, Sinaloa, Mexico (26° 06’ 15.35” north latitude, 109° 33’ 74.65” west longitude). The period of the work was from June 2018 to April 2019; during this period, 12 successive monthly applications of imidacloprid were made on immature individuals of the Asian citrus psyllid and the biological effectiveness of this insecticide was evaluated (Table 1).

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Table 1. ates of spraying of imidacloprid on immature ACP in a commercial Persian lime grove.

Treatment	Spraying date	Treatment	Spraying date
1	25/06/2018	7	21/11/2018
2	25/07/2018	8	24/12/2018
3	18/08/2018	9	11/01/2019
4	11/09/2018	10	08/02/2019
5	06/10/2018	11	11/03/2019
6	26/10/2018	12	01/04/2019

Three rows 200 m long were used, the central row was considered as a useful plot, eliminating the first two trees from the front and back of the end of the row, which was done to eliminate the edge effect because unwanted factors may intervene in the treatments. Before each application, 18 tender shoots (shoots less than 5 cm in length, which had leaves of a lighter green color than the rest of the foliage, succulent-looking) with the presence of *D. citri* were labeled, which were distributed in the 18 trees of the central row of each treatment (one shoot/tree).

In these shoots, the number of eggs, small nymphs (from nymph 1 to nymph 3, less than one mm in length), and large nymphs (from nymph 4 to nymph 5, larger than one millimeter in length) was counted and the data was recorded in a field book and on a plastic label placed at the bottom of each shoot, which served to locate each inspected shoot, said sampling was carried out one day before applying the treatments. At the time of the initial count, adults were discarded (removed with a brush) from confinement because they could oviposit and alter the results.

Insecticide applications were made with an Echo® SHP-800-2 engine backpack sprayer, with two spray wands with D-5 double-orifice nozzles (acceleration of 200 pounds of force per square inch, PSI). Before depositing the insecticide in the water of the backpack sprayer tank, one ml L^{-1} of acidifying adjuvant (Surfacid®) water was added to regulate the pH and prevent alkaline hydrolysis of the insecticide.

Then the insecticide was added at a dose of 300 ml ha^{-1} and the mixture was homogenized by stirring vigorously, then the rest of the water was added and the applications were made in the morning. Subsequently, the inspected tender shoots were confined with an agribon net to prevent external factors (mainly predators) from intervening on the mortality of immature ACP individuals.

At 72 h after the application of the treatments (AAT), the shoots of the previous sampling were inspected again and the specimens present were counted. In the end, 12 applications were made at approximately 25-day intervals; each shoot per tree was considered a replication (n= 18) and each application was considered as a treatment. The variables evaluated were the total number of live immature individuals of *D. citri* (eggs, small and large nymphs) in tender shoots in previous samplings and at 72 h AAT.

The percentage of mortality in each shoot and each replication was obtained by difference in the number of specimens. Mortality data at each stage of insect development were subjected to an analysis of variance (Anova) using a completely randomized design (CRD) and a comparison of means by Tukey (α= 0.05).

Results and discussion

In the Anova of the biological effectiveness of imidacloprid on *D. citri* eggs, there was a significant difference; the populations of the treatments showed different distribution in some of the treatments; in the comparison of means, it was determined that the highest mortality was obtained (74.96%) on the first date of application (June 25, 2018) and it was significantly different ($p< 0.05$) from the tenth, eleventh, and twelfth treatments (Table 2).

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Table 2. Biological effectiveness of imidacloprid on ACP on 12 spraying dates 72 h AAT.

Date of application	Average percentage of mortality		
	Eggs	Small nymphs	Large nymphs
1. 25/06/2018	74.96 A*	88.62 A*	85.77 A*
2. 25/07/2018	71.18 A	89.63 A	83.28 A
3. 18/08/2018	74.89 A	85.97 ABC	82.47 AB
4. 11/09/2018	72.28 A	90.66 A	80.61 ABC
5. 06/10/2018	73.16 A	84.27 ABCD	75.15 ABC
6. 26/10/2018	64.79 A	84.23 ABCD	78.68 ABC
7. 21/11/2018	64.54 A	69.67 D	75.44 ABC
8. 24/12/2018	60 AB	71.33 CD	72.83 ABC
9. 11/01/2019	65.87 A	80.06 ABCD	78.73 ABC
10. 08/02/2019	40.27 C	73.57 BCD	71.49 ABC
11. 11/03/2019	42.98 BC	70.47 D	67.59 BC
12. 01/04/2019	37.78 C	76.45 ABCD	65.91 C

* = data with different letters are significantly different (α= 0.05).

The results allow us to clarify that the susceptibility of the egg stage of the ACP decreased significantly after nine repeated applications of imidacloprid. From the sixth application of the insecticide, the reduction in ACP susceptibility decreased compared to previous applications (about 10%) and from the tenth date of spraying, it declined by more than 30%.

In the Anova of the biological effectiveness of the insecticide on small nymphs, there was a significant difference, the populations of the treatments showed different distribution in some of the treatments; in the comparison of means, it was determined that the highest mortality was obtained on the fourth date of application (September 11, 2018), with 90.66% (Table 2), and it was significantly different ($p< 0.05$) from the seventh, eighth, ninth, tenth, and eleventh treatments.

The above results allow us to clarify that the susceptibility of the small nymphs declined significantly after six repeated applications of imidacloprid. The reduction in mortality was 10% from the seventh date of application while on the tenth date of spraying, mortality decreased by 17%.

In the Anova of the biological effectiveness of the insecticide on large nymphs, a significant difference was observed, the populations of the treatments showed different distribution in some of the treatments; in the comparison of means, it was determined that the highest mortality was obtained on the first date of application (June 25, 2018), with 85.7% (Table 2); this treatment was significantly different from the eleventh and twelfth treatments.

That is, the treatments applied on the last two dates showed lower mortality on large nymphs. The reduction in mortality was 10% from the seventh date of application and from the eleventh date of spraying, mortality declined by 18%. The first four spraying dates showed the highest mortality (> 80%). Figure 1 shows the mortality assessed in three stages of ACP development and exhibits a similar mortality trend between eggs, small and large nymphs.

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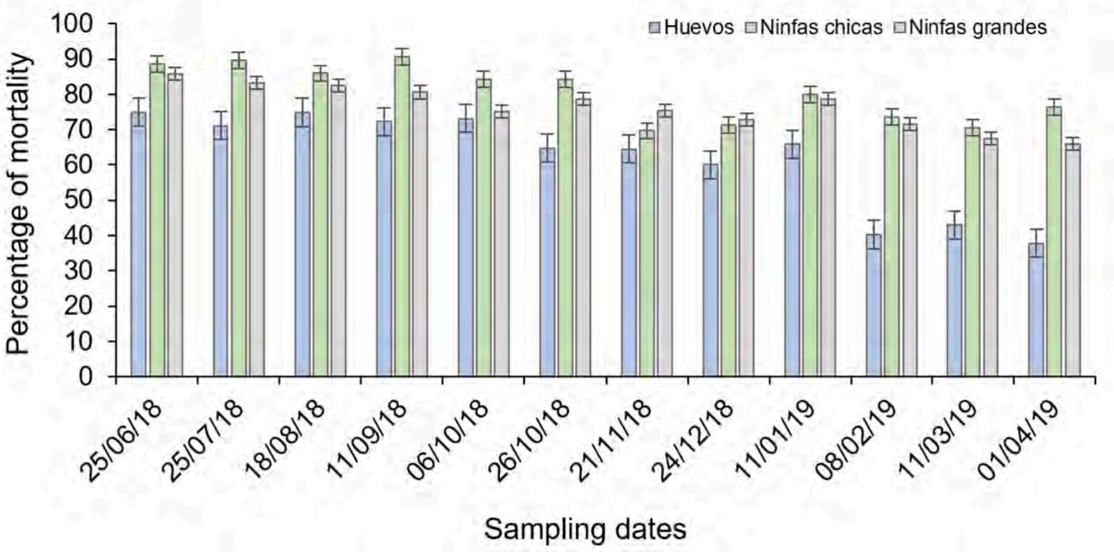
Figure 1. Biological effectiveness of imidacloprid on ACP on 12 spraying dates.



Mortality in these three stages of ACP development was higher on the first four dates of application (82.2%), which were from June 25 to August 11, 2018. On the contrary, the lowest mortality was recorded on the last three dates, from February 8 to April 1, 2019 (65.7%). On the other five dates of application of the treatments (from October 6, 2018, to January 11, 2019), mortality was approximately 71.7%.

In accordance with what was reported by Ramírez *et al*. (2018), in this work, mortality was observed in the egg stage due to the effect of the application of imidacloprid; another work conducted by Ruíz-Galván (2013) reported that this insecticide significantly delayed egg hatching (63%) and when they emerged, all the nymphs died in less than 24 h. The eggs at 72 h AAT were dehydrated and sometimes the apical part showed a necrotic coloration; this is probably due to the contact effect of the insecticide. However, mortality was lower than in nymphs.

Between the small and large nymphs, there was no notable difference in the percentage of mortality, but it was slightly higher in the small nymphs, where the average mortality on all spraying dates was 80.4% while in the large nymphs, it was 76.5%. In other studies, higher mortality has been observed in small nymphs compared to other stages of development of this insect (Díaz, 2010; Cortez *et al*., 2013), which indicates that young nymphs are more susceptible to insecticides due to body size and weight (Alemán *et al*., 2007; García-Méndez, 2013).

It is important to mention that imidacloprid showed a high biological effectiveness (from 80%) in the first six dates of evaluation of the insecticide on small nymphs, with a mortality of 87.2%, while mortality for large nymphs was 80.1%. The highest percentage of egg mortality (75%) of the ACP was recorded on the first spraying date (June 25, 2019) whereas the lowest percentage (37.8) was recorded on the last spraying date (April 1, 2019).

In the case of small nymphs, the highest mortality recorded was in the fourth spraying (90.66%), which was carried out on September 11, 2018, while the lowest mortality (69.7%) occurred in the seventh spraying (November 21, 2018). On the other hand, the highest mortality of large nymphs was 85.8% and was observed on the first date whereas the lowest mortality (65.9%) was recorded on the last date of spraying.

For the reasons mentioned above, it is possible to use it indefinitely when the number of successive applications is less than four per season and if they are carried out in rotation with insecticides from different groups of mode of action and with different sites of action (IRAC, 2022). The competitive modulators of nicotinic acetylcholine receptors are those that confer resistance to insects selected by the insecticides that make up group 4 (IRAC, 2022).

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When these insecticides are used indiscriminately, resistant specimens are selected, as occurred in Guangdong, China, where Fajun *et al*. (2018) mentioned high resistance levels of *D. citri* to imidacloprid [resistance ratio (RR)= 15.12] compared to other insecticides such as dinotefuran, chlorpyrifos, thiamethoxam, lambda-cyhalothrin, and bifenthrin.

Likewise, in Punjab, Pakistan, a similar study conducted by Naeem *et al*. (2016) reports that *D. citri* populations were highly resistant to this insecticide (RR= 236.6 - 759.5) compared to chlorpyrifos, bifenthrin, acetamiprid, thiamethoxam, chlorfenapyr, and nitenpyram. On the other hand, in a study conducted in the laboratory in Florida (USA) by Langdon and Rogers (2017), they mentioned that the biological effectiveness of imidacloprid on four different populations of *D. citri* was greater by ingestion than by contact, so the application of the insecticide in the pressurized irrigation system should be promoted (Cortez *et al*., 2013).

In a study similar to the present research, conducted by Della *et al*. (2019), they made some mixtures of chemical insecticides (lambda-cyhalothrin, thiamethoxam, fosmet, and imidacloprid) to evaluate the biological effectiveness on *D. citri*; these same insecticides were also evaluated individually and no significant differences were found between the mixed treatments compared to the unmixed treatments (mortality remained around 80% for both cases). This mortality is consistent with that reported in the present study.

The two applications of insecticides in ARCOs with which the Mexican government, through the General Directorate of Plant Health (DGSV, for its initialism in Spanish), supports citrus producers annually in the country can be carried out for an indefinite period of time in the state of Sinaloa and others where there is no resistance (SENASICA, 2017). On the contrary, where resistance has already been determined, the insecticide should be discontinued indefinitely (Tiwari *et al*., 2013; Vázquez *et al*., 2013).

Several studies similar to the present one, conducted with different insecticides used to combat ACP, provide information to define, in field conditions, the viability of their effectiveness over time, without running the risk of selecting resistant populations that are reflected in control failure. In this sense, there would be many benefits that would be achieved, such as ceasing to use chemical insecticides with little biological effectiveness, insecticides that have the same mode and site of action, insecticides that are more economically profitable and that have the same or superior biological effectiveness, etc.

Conclusions

The biological effectiveness of imidacloprid on eggs and small and large nymphs of the Asian citrus psyllid was high in several successive initial applications (80%); in contrast, mortality on eggs declined by 10% from the sixth successive application of the insecticide and after the tenth date of spraying, mortality decreased significantly by more than 30%; the reduction in mortality of small nymphs was 10% from the seventh date of application and after the tenth date of spraying, mortality was significantly lower by 17%; the reduction in mortality of large nymphs was 10% from the seventh date of application and after the eleventh date of spraying, mortality declined significantly by 18%.

The percentages of mortality reduction among the sampling dates ranged between 10 and 30% in eggs and small and large nymphs; this decrease can be considered between slight and moderate, which would indicate the beginning of resistance of the insect to the insecticide. It is necessary to carry out new studies in order to evaluate the biological effectiveness of new generation insecticides with different modes and sites of action against immature individuals of *D. citri* in commercial Persian lime groves in order to determine which insecticides can be used in the regional control areas (ARCOs) to have a greater number of insecticidal molecules that can be used against *D. citri* and thus have effective control over the HLB insect vector. It is also suggested to verify the biological effectiveness of chemical insecticides on ACP adults in order to have a better picture of the effect of the insecticide in field conditions.

DOI: https://doi.org/10.29312/remexca.v16i2.3409

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DOI: https://doi.org/10.29312/remexca.v16i2.3409

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Biological effectiveness of imidacloprid on the Asian citrus psyllid

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher:

Article/Issue Information
Date received: 01 January 2025
Date accepted: 01 April 2025
Publication date: 08 April 2025
Publication date: Feb-Mar 2025
Volume: 16
Issue: 2
Electronic Location Identifier: e3409
DOI: 10.29312/remexca.v16i2.3409

Categories

Subject: Articles

Keywords:

Keywords:

chemical control
insecticide
mortality

Counts

Figures: 1
Tables: 2
Equations: 0
References: 38
Pages: 0